

Final Report Summary

		NOT CONFIDENTIAL
Title of the project Evaluating human health risk from low-dose and long-term PCB exposure		
Acronym of the project PCBRISK		
Type of contract shared cost	Total project cost €2,081,000	
Contract number QLK4-CT-2000-00488	Duration 42 Months	EU contribution €1,130,000 €
Commencement date March 1, 2001	Period covered by the report March 1, 2001 - August 31, 2004	
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Key words Human exposure, Health risk, PCBs		
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Section 2: PROJECT PROGRESS REPORT**NOT CONFIDENTIAL**

Objectives: The overall objective of this project is to evaluate the human health risks of low-dose and long-term exposure to a group of persistent organochlorine pollutants, including polychlorinated biphenyls (PCBs) and their metabolites, organochlorine pesticides, poly-chlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) within a population that has been exposed to these chemicals as a result of environmental pollution.

In connection with this main objective the following activities are planned:

- To evaluate the health effects of human exposure to persistent organic pollutants, including their carcinogenic potential.
- To assess the long-term endocrine-disrupting (thyroid, gonads and reproduction) and immunomodulatory effects of exposure to PCBs and related compounds.
- To examine neurotoxic effects of PCBs and organochlorine pesticides in children considering heavy metals as confounders.
- To evaluate the dioxin-like and estrogenic activity of PCBs and related compounds present in human blood.
- To contribute to the validation of a method for estimating total dioxin and dioxin-like toxic equivalents (TEQ) using the Calux bioassay as a surrogate for the costly classical chemical analysis.
- To provide data to the pan-European databases relevant to exposure to persistent organic pollutants.
- To strengthen the knowledge base on health effects of persistent organic pollutants as the basis for informed policy-making

Results and Milestones:

In Slovakia, the Chemko Chemical Company based in Strazske, in the Michalovce district, produced PCBs between 1959 and 1984, in the amount of 21,000 tons of commercial mixtures (Delor 103, 104, 105, 106, Delotherm DK and DH, Hydeler 137). Improper disposal from the Chemko plant via release of effluent directly into the Laborec River resulted in long-term contamination of sediment. As a result eastern Slovakia, the Michalovce district in particular, is recognized as one of the areas all over the world most heavily polluted with PCBs. Current data indicate that persons who eat locally raised food - pork, beef, poultry, eggs - in this district have elevated serum concentrations of PCBs. This "experimental setting in nature" has attracted international scientific teams and besides the PCB RISK project in the area is ongoing the project "Early Childhood Development and PCB Exposures in Slovakia, NCI/NIH, # R01-CA96525 University of California, Davis, USA (<http://slovakchildren.ucdavis.edu/index.php>).

The overall objective of PCB RISK was to evaluate the human health risks of low-dose and long-term exposure to a group of persistent organochlorine pollutants, including polychlorinated biphenyls (PCBs) and their metabolites, organochlorine pesticides, poly-chlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) within a population that has been exposed to these chemicals as a result of environmental pollution. The following activities were planned: To evaluate the health effects of human exposure to persistent organic pollutants, including their carcinogenic potential. To assess the long-term endocrine-disrupting and immunomodulatory effects of exposure to PCBs and related compounds. To examine neurotoxic effects of PCBs and organochlorine pesticides in children considering heavy metals as confounders. To evaluate the dioxin-like and estrogenic activity of PCBs and related compounds present in human blood. To contribute to the validation of a method for estimating total dioxin and dioxin-like toxic equivalents (TEQ) using the Calux bioassay as a surrogate for the costly classical chemical analysis. To provide data to the pan-European databases relevant to exposure to persistent organic pollutants. To strengthen the knowledge base on health effects of persistent organic pollutants as the basis for informed policy-making. The project was a cross-sectional epidemiological study and consisted of the following elements: Examination of 2478 subjects (2047 adults, 50.7% females and 49.3% males, and 431 8-9 years old children) living in a PCB-polluted area. In all subjects blood samples were analysed for PCBs and organochlorine pesticides, hormones, antibodies, biomarkers and questionnaires on biomedical data were completed. In all adults with no contraindication (1200) was performed the oral glucose tolerance test. In 300 of the adult subjects were done additional estimations of OH-PCBs, MeSO₂-PCBs, PCDDs, PCDFs, coplanar PCBs, metals (Cd, Hg, Pb, Mn, Zn, Se), bioassays of dioxin-like activity and xenoestrogenic activity. In 431 children were examined cognitive and hearing functions, dental status and organochlorine and toxic metal levels in serum and thyroid morphology and function. Sampling from an area approximately 80 km by 40 km, excentric to the north from the source of pollution in Strazske, with over 300000 permanent residents, gave the following data for sum of PCB serum concentration (ng/g serum lipids): for 2047 adults mean 1955.1, median 1065, limits of the quartiles 124, 675, 1065, 1978, 101411; for 433 8-9 years old children mean 528.2, median 321, limits of the quartiles-18, 175, 321, 618, 6477. Even the lognormal display of frequency distribution of PCB serum concentrations was skewed toward higher concentrations. Out of the sum of all the congeners analyzed, #153 and the sum of #153, 138⁺¹⁶³ and 180 were representing on average 30.6% ($s_x = 2.3\%$) and 77.5% ($s_x = 3.0\%$), respectively.

Annex I: Project Progress Summary

PCBs formed hydroxylated (OH-PCBs) and methylsulfonyl substituted metabolites (MeSO₂-PCBs). The humans from the Michalovce have the hitherto highest OH-PCB levels determined in the world. It was possible to quantify at least one PCB methyl sulfone in all 319 subjects. The major congeners were 4-MeSO₂-CB149 and 4-OH-CB-187. The 4-OH-CB187 is a metabolite of CB-183 and CB-187, the former going through a 1,2-shift to form 4-OH-CB187.

Relationships between exposure to PCBs and decrease of E2 levels and overall (anti)estrogenic activity were significant at high exposure levels. High PCB levels in serum could contribute to antiestrogenic effects. The prevalent noncoplanar PCBs, as well as PCB metabolites, elicited antiestrogenicity in the ER-CALUX assay, when tested as individual compounds or as a partially reconstituted mixture. Due to the presence of E2 in human male blood and its dominant role in total estrogenic activity of serum samples, reduction of E2 levels appears to be the most significant antiestrogenic effect of high PCB exposure. Induction of CYP1A1, CYP1A2, CYP1B1 and CYP3A4 by PCBs, enzymes responsible for metabolic inactivation of E2, could play a role in this effect. Since the data on estrogenicity and E2 concentrations were negatively correlated with CYP1A1 and CYP1B1 mRNA expression, modulation of blood concentrations of E2 by PCB-induced metabolism of E2, might play a more significant role in endocrine disruption than the direct effects on the ER-mediated activity.

Dioxin-like activity determined in the serum of males by the DR-CALUX assay was significantly increased only in subjects with high PCB exposure. The data cannot fully support the applicability of CYP1A1 or 1B1 gene expression in blood lymphocytes as biomarkers for background TEQ exposure levels in concentration range studied. The higher PCB exposure of the population from the Michalovce district does not influence Ah receptor mediated CYP1A1 and CYP1B1 gene expression compared with that of the less polluted area. Polymorphism of CYP1B1 in the populations studied was normal and did not have an effect on its expression in blood lymphocytes.

Few samples of blood, serum or urine had increased concentrations of toxic elements, and none were at a level consistent with risk of significant exposure. The exposure by the children is within generally accepted levels. There does not appear to be any relation of trace element concentrations to the subject groups or to serum PCB concentrations. One exception is urine uranium.

In all subjects the thyroid volume examined by ultrasound was increasing with PCB serum level. Thyroid hypoechoogenicity and level of anti-thyroperoxidase autoantibodies in blood was increased in subjects with increased PCB level indicating autoimmune impairment of the thyroid. The levels of free thyroxine and total triiodothyronine were increasing with PCB level thus possibly showing impairment of equilibrium between bound and free thyroxine in plasma due to the displacement of thyroxine from plasma protein binding by high levels of PCB further resulting in increased rate of thyroxine to triiodothyronine conversion in peripheral tissues. The frequency of increased levels of thyrotropin was about twice as high in females than in males, not showing any considerable interrelations with increasing PCB level. However, the frequency of decreased thyrotropin levels was increased in the upper quintile of PCB levels in both males and females. Incidence of diabetes and other dysglycemias was related to serum concentrations of PCBs and other pollutants. The serum levels of PCBs, HCB, HCHs, DDT and DDE were associated with each other, suggesting simultaneous exposure. In females, predictive parameters for fasting plasma glucose, besides age and BMI, were concentrations of HCB, β HCH and DDT and in males BMI, β -HCH and age. The predictive parameters for fasting plasma insulin, besides BMI, were DDT and DDE for females and males, respectively.

The levels of tumour markers (e.g. carcinoembryonic antigen, alpha-fetoprotein and β_2 -microglobulin) in 99.4-99.8 % of 2040 individuals showed normal levels, the rest was slightly above the lower limit of positive level, most of subjects with very high levels of PCB.

PCBs serum concentrations in children were related to breastfeeding, its length and maternal age. In breast-fed children, the parameters of sensomotor tests, Benton memory test and behavioral scales correlated with PCBs serum levels and were associated with age, gender, child birth weight and education of mother. Hyperactivity at home was related with both, serum PCBs and blood lead levels, but hyperactivity at school was associated with serum PCBs levels and with the age and gender of children. After adjustment for the variable "school", there were significant associations among PCB serum concentrations and performances in the sensomotor tests, breastfeeding, age and gender of children, education of mother and locations of schools. Results in higher mental tasks were significantly associated with the age of children, maternal intelligence level and education, and school location, but not with serum PCBs levels. Recurrent middle ear infections in children were frequently accompanied by a decreased middle ear pressure. An association between negative middle ear pressure and PCB serum concentrations was found. Increases of the hearing thresholds at low frequencies were associated with quartiles of PCB serum concentrations. There were no associations between the serum PCB concentrations and any of the auditory brainstem response parameters measured. Increasing PCB serum levels were associated with decreasing emission level of the transient evoked otoacoustic emissions. The simple reaction time was positively associated with the hearing threshold. The sound pressure level of transient evoked otoacoustic emissions was associated with results of all neurobehavioral tests applied except Raven intelligence test. A correlation was found between the sound pressure level of the transient evoked otoacoustic emissions and proportion of permanent teeth with demarcated, diffuse opacities or hypoplasia.

A correlation was found between the PCB serum concentrations and percentage of permanent teeth with various types of enamel defects: demarcated and/or diffuse opacities, hypoplasia.

PCBs or PCDFs do not appear to be associated with observed lower incidence of breast and possibly prostate cancer in the Michalovce district. Nevertheless, a moderate contribution of antiestrogenic compounds (both dioxin-like compounds and non-dioxin-like PCBs and PCB metabolites) might be expected.

Benefits and Beneficiaries:

The immediate beneficiaries are the subjects medically examined in whom were discovered some previously unrecognised illnesses. Namely in "healthy" volunteers several cases of diabetes, thyroid gland pathologies, hearing impairment, etc. were diagnosed. The ultimate benefit from the study will be a contribution to the knowledge of mechanisms of action of organochlorine compounds.

Future Actions (if applicable):

Publishing results